(54) Title: A METHOD FOR PREPARING AN ORGANIC ACID OR ITS SALT

(57) Abstract

The invention relates to a method for preparing an organic acid or its salt by a continuous process. In accordance with the invention, a feed solution is continuously passed into a bioreactor containing microorganisms bound to a solid carrier, the acidic solution withdrawn from the bioreactor is passed through a column of an anion exchanger regenerated with alkali metal hydroxide, the feed solution withdrawn from the anion exchange column is recycled to the bioreactor, and at suitable intervals, the feed solution is displaced by water and the anion exchange resin is regenerated with alkali metal hydroxide to recover the acid as an alkali salt. Finally, if acid is the desired end product, the alkali metal salt solution is passed through a column of a cation exchanger in hydrogen ion form to yield an acid.

1

A method for preparing an organic acid or its salt

The present invention relates to a method for
preparing an organic acid or its salt by a continuous
5        process.
The method of the invention is characterized in
that
a feed solution is continuously passed into a
bioreactor containing microorganisms bound to a solid
10       carrier,
the acidic solution withdrawn from the bioreactor
is passed through a column of an anion exchanger regen-
erated with alkali metal hydroxide, to which column the
acid is bound,
15       the feed solution withdrawn from the anion ex-
change column is recycled to the bioreactor, and
at suitable intervals, the feed solution is dis-
placed by water and the anion exchange resin is regen-
erated with alkali metal hydroxide to recover the acid
20       as an alkali salt,
whereafter, if acid is the desired end product,
the alkali metal salt solution is passed through a col-
umn of a cation exchanger in hydrogen ion form to yield
an organic acid.
25       Normally, an attempt is made to make the conver-
sion of raw material into an acid as complete as pos-
sible to minimize raw material costs and obtain a prod-
uct with maximum purity. On the other hand, when the
conversion is improved the reaction is retarded on
30       account of the pH decrease, for instance. To improve
the conversion, it is known to recycle the majority of
the product solution to the reactor subsequent to pH
adjustment; only a small part of the product solution
is passed to product recovery. It is also possible to
35       accept lower conversion, to concentrate the product

2

solution after separation of the acid, and to reuse the raw material contained in the solution.

Furthermore, the acid formation reaction is somewhat end product-inhibited as regards the acid formed, which thus also retards the reaction. It is known to reduce end product inhibition by adding into the bioreactor a suitable adsorbent, such as an anion exchange resin, to which the acid produced is bound. Likewise, it is known to recover the acid from the solution by ion exchange.

In the method of the present invention, the novelty lies in combining continuous bioconversion (with microorganisms bound to a solid carrier) with continuous separation of the resulting acid (by ion exchange) and pH regulation of the solution (by ion exchange) into a process in which the feed solution is continuously recycled from a bioreactor comprising immobilized microorganisms to an anion exchanger and directly back to the reactor. The anion exchanger, comprising at least two ion exchange columns, serves as a 'carousel' in which the columns undergo the cycle adsorption - displacement - regeneration - washing - adsorption, etc. Feed solution is continuously supplied to the process at the same rate at which acid is produced in the reactor and bound to the anion exchanger.

The advantages of the method of the invention over the prior art are the following:

End product inhibition is minimized, since acid is removed from the whole stream to be recycled, instead of only part of the outflow of the bioreactor being recovered as a product fraction.

Since each produced/removed acid residue liberates one hydroxyl ion in the anion exchanger, the resulting protons are neutralized into water, and pH inhibition is minimized without actual pH adjustment.

3

The productivity of the bioreaction can be maximized without maximizing the conversion, since unreacted raw material is recycled to the bioreactor.

It is not necessary to maximize the acidity of the bioreactor outflow to save costs, since the acid, i.e. the product, is similarly concentrated in the ion exchanger regardless of the starting concentration.

Useful anion exchange resins include both strong and weak type anion exchange resins. What is essential is that the resin is capable of binding acid (the acid ion) in a suitable pH range. Such resins include Amberlite IRA 900 (Rohm & Haas), Dowex MSA-1 (Dow Chemicals) and Relite 3 AS (Mitsubishi).

The method of the invention is capable of producing water-soluble, natural carboxylic acids obtainable by bioconversion, such as itaconic acid, fumaric acid, gluconic acid, citric acid, maleic acid and lactic acid. When acid of a very high purity is to be produced, it is preferable to employ a raw material that is free of other acids. Acids may be removed from the raw material by pretreating the feed solution in an anion exchanger.

Suitable microorganisms include natural and/or selected microorganisms, or those produced by adaptation, or those mutated to produce the desired organic acid.

The bioreactor may be for example a mixed tank, basket, fluidized bed, packed bed, or filter reactor.

Thus the process of the invention can continuously produce lactic acid, for example, with microorganisms, such as lactic acid bacteria, immobilized to a fixed bed reactor, for instance. Sugar, e.g. glucose, lactose, sucrose or xylose, or a sugar-containing solution, such as molasses, is preferably employed as the raw material. The raw material-containing solution is

4

continuously passed into a bioreactor comprising mic-
roorganisms that produce lactic acid and are bound to
a solid carrier. The lactic acid-containing solution
withdrawn from the bioreactor is passed through a col-
5        umn of an anion exchanger, to which column the acid is
bound. As the capacity of the ion exchange column is
exhausted, the column is emptied of solution by dis-
placement with water and disconnected from the cycle
for regeneration. In that connection, another, regener-
10       ated ion exchange column is connected to the cycle.
Regeneration is preferably performed with a sodium
hydroxide solution, enabling the resin to become regen-
erated into the hydroxyl form and lactic acid to be
recovered as sodium lactate. If desired, the sodium
15       lactate is further passed through a cation exchanger in
the hydrogen ion form, wherefrom the product is recov-
ered as lactic acid.

It is known to produce lactic acid continuously
by means of bacteria bound to a solid carrier and also
20       by normal bioconversion. When lactic acid is produced
the pH of the solution decreases, which will slow down
the reaction. It is known to prevent retarding of the
reaction induced by the decrease in pH by adding a
base, e.g. a sodium hydroxide solution, to the solu-
25       tion, and normally an attempt is made to make the
conversion of sugar into lactic acid as complete as
possible to minimize raw material costs and obtain a
product of maximum purity. To improve the conversion,
it is known to recycle the majority of the product
30       stream subsequent to pH adjustment back to the reactor;
only a small part of the product stream is passed to
product recovery. It is naturally also possible to
accept lower conversion, to concentrate the solution
after separation of lactic acid, and to reuse the sugar
35       contained in it as a raw material.

5

Furthermore, the formation reaction of lactic acid is somewhat end product-inhibited as regards the acid, which thus also retards the reaction. It is known to reduce end product inhibition by adding a suitable adsorbent, such as an anion exchange resin, into the reactor, the resulting acid binding to the resin. Likewise, it is known to recover the acid from the solution by ion exchange.

In the method of the present invention, the novelty lies in combining continuous bioconversion (with microorganisms bound to a solid carrier) with continuous separation of the resulting acid (by ion exchange) and pH regulation of the solution (by ion exchange) into a process in which the feed solution is continuously recycled from a bioreactor comprising immobilized microorganisms to an anion exchanger and back to the reactor. The anion exchanger, comprising at least two ion exchange columns, serves as a 'carousel' in which the columns undergo the cycle adsorption - displacement - regeneration - washing - adsorption, etc. Feed solution is continuously supplied to the process at the same rate at which acid is produced in the reactor and bound to the anion exchanger.

When the anion exchange resin is regenerated with sodium hydroxide, the product is recovered as sodium lactate, suitable as such for several applications. Potassium hydroxide regeneration respectively yields potassium lactate.

If specifically lactic acid is the desired end product, the alkali metal lactate is further passed into a cation exchanger charged with resin in the hydrogen ion form, operating on the same carousel principle as the anion exchanger.

It is essential in the method of the invention that the bioconversion is performed continuously, em-

6

ploying immobilized microorganisms. A packed column
reactor is preferably used. The microorganisms are
bound to a carrier material having a large surface
area. The carrier is preferably substantially non-
compressible and preferably comprises a continuous
matrix having a large surface area or, alternatively,
porous or reticular grains having a large surface area.
The matrix or grains again comprise discrete micro-
particles or microfibres. Such a carrier material
texture affords maximum surface area in view of the
immobilization of the bacterial cells. A granular or
matrix structure is produced when the microparticles or
microfibres are bound, compacted, interwoven, adhered
or agglomerated together (hereinafter bound together).
The binding takes place by means of chemical, adhesive
or mechanical bonds between certain contact points of
the discrete microparticles or microfibres.

The microfibres or microparticles may comprise
any anion exchanging agent wherefrom rough microfibres
or microparticles can be formed. These substances in-
clude native or regenerated cellulose or rayon, to
which an anion exchanger effect has been imparted by
derivatizing, synthetic anion exchanger resins, such as
phenol formaldehyde resin and agarose-based and dex-
trin-based anion exchanger resins. A preferred carrier
material is a porous granular anion exchanger resin: a
cellulose or rayon derivative chemically modified so as
to impart to it an anion exchanger effect. According to
a particularly preferred embodiment, the carrier mater-
ial comprises microfibres or microparticles of diethyl-
aminoethylene-substituted cellulose (DEAE cellulose)
adhesively bonded with agglomeration to polystyrene
(cf. U.S. Patent 4 355 117). Other suitable agglomer-
izing substances include melamine formaldehyde resin
and e.g. epoxy resins (cf. DE 31 30 178 C2).

7

Porous, sintered glass or a ceramic material may also be employed as the carrier.

The bioreactor of the invention has a very high content of bound cells. The cells are very strongly bound, and hence the solution withdrawn from the bioreactor to recovery contains essentially no microorganism cells.

The bioreactor may be regenerated by displacing the feed solution from the reactor with hot water and treating the carrier with hot lye, until the regenerant outflow has a uniform light colour. Thereafter the carrier is flushed with water until the pH is about 10, which is followed by neutralization with a suitable dilute acid. Finally the carrier is again flushed with water.

In the following, the method of the invention will be explained in more detail, applied to the production of lactic acid.

A solution containing raw material, preferably glucose, and nutrients is pumped into a bioreactor, preferably into a packed bed reactor comprising microorganisms bound to a solid carrier.

Suitable microorganisms include, in the first place, lactic acid bacteria, such as lactic acid bacteria of the genera Aerococcus, Carbobacterium, Enterococcus, Erysipelothrix, Gemella, Globicatella, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus and Vagococcus. Also moulds, such as Rhizopus moulds, may be employed.

Particularly preferred microorganisms include *Lactobacillus delbrückii*, *Lactobacillus bulgaricus* and *Lactobacillus leichmanii*, and *Rhizopus oryzae*.

Also other hexoses than glucose, or substances easily convertible to hexoses (glucose) may be employed as the raw material. In other words, suitable raw

8

materials include sugar molasses, diffusion juices and
starches.

The lactic acid-containing solution withdrawn
from the bioreactor is passed, if necessary, into a
5       purifier unit in which the solution is clarified, and
thereafter to an anion exchanger in which the acid is
bound as lactate ions with simultaneous release of
hydroxyl ions.

Useful anion exchange resins include both strong
10      and weak type anion exchange resins. What is essential
is that the resin is capable of binding the lactate
ions in the pH range 3.0-7.0. Such resins include
Amberlite IRA 900 (Rohm & Haas), Dowex MSA-1 (Dow Chem-
icals) and Relite 3 AS (Mitsubishi).

15      The anion exchanger preferably comprises several
anion exchange columns, for example 2 to 4 columns.
When a lactic acid-containing solution is passed
through one column, another column is being regener-
ated. Thus in practice the anion exchange process oper-
20      ates continuously. If necessary, the solution withdrawn
from the anion exchange column is passed into a buffer
vessel in which the pH is fine tuned.

Thus a column or column system of a fixed size
can be operated in practice in a continuous cycling
25      system with bioreactors of varying sizes and flow
rates.

The following examples illustrate the invention.
However, the working examples are not to be construed
as limiting the invention, but the characteristic
30      features of the invention are disclosed in the claims.

Example 1
Construction of a packed column reactor
Granular DEAE cellulose (GDC) prepared by Cultor
35      Oy in accordance with U.S. Patent 4 355 117, having a

9

particle size of 350-850 µm, was employed as a carrier. The column was filled, the system sterilized and the bacteria immobilized in accordance with the procedure set out below.

5     80 g (200 ml) of granular DEAE cellulose were suspended in distilled water, and the suspension was mixed periodically for 5 hours. The hydrated carrier was transferred to a glass column with an inner diameter of 50 mm and height of 150 mm. The bed height was
10   100 mm. Prior to immobilization, the bed was sterilized with 70% ethanol and flushed with distilled water.

*Lactobacillus delbrückii* was cultivated for 2 days in MRS broth. 800 ml of this culture were passed at 40°C through the resin bed at a low flow rate, which
15   was followed by passage of 500 ml of distilled water. About $2.5 \times 10^9$ bacteria/g dry carrier were bound to the carrier.

Bioconversion

The reaction temperature was 42°C. A feed solution
20   was passed through the biocolumn at a rate of 2 l/h (10 bed volumes/h). The feed solution had the following composition:

|                    |                    |
|--------------------|--------------------|
| glucose            | 10 g/l             |
| yeast extract      | 1 g/l              |
| 25 phosphate buffer | 1.7 g/l (pH = 6.5) |
| magnesium chloride | 0.1 g/l            |
| manganese chloride | 0.1 g/l            |

The productivity of the bioreactor was 12 g
30   lactic acid/l reactor volume/h. The reactor was monitored for 6 weeks.

10

Example 2

Separation of lactate

Two parallel anion exchange columns having a
height of 150 mm and a diameter of 50 mm were used,
5   each containing 200 ml of a strong anion exchange
resin, Relite 3 AS (Mitsubishi). One of the columns was
in operation stage and the other in regeneration stage.
The operation was controlled by a microprocessor.

A bacteria-free solution containing 1.2 g/l of
10  lactic acid was passed through the first column, which
had been regenerated with a 4% (w/w) sodium hydroxide
solution, at a flow rate of 2 litres/hour. After about
4 hours (when the pH of the effluent had decreased to
6.0) the feed solution was directed to the other col-
15  umn, and the feed of the first column was changed to
water (250 ml; about 7 min). After that, 200 ml of 4%
(w/w) of a sodium hydroxide solution were passed
through the column at a low flow rate, which yielded a
basic solution of sodium lactate. Thereafter the column
20  was flushed with 400 ml of water to recover the lactate
tailings, with a final backwash by upflow with 600 ml
of water. The resin was allowed to settle, after which
the column was ready for another operating cycle.

Example 3

25  Recovery of lactic acid

Two parallel cation exchange columns having a
height of 150 mm and a diameter of 50 mm were used,
each containing 200 ml of a strong cation exchange
resin, Relite C 360 (Mitsubishi). One of the columns
30  was in operation stage and the other in regeneration
stage. The operation was controlled by a micropro-
cessor.

A basic sodium lactate solution (600 ml; sodium
lactate content 16 g/l) was passed through the first
35  column - which was in the hydrogen ion form - at a rate

11

of 150 ml/h, and a dilute lactic acid solution was re-
covered. The dilute lactic acid was concentrated by
evaporation.

Water was passed through the said first column
(400 ml; about 1 h). Thereafter, 280 ml of 5% hydro-
chloric acid were passed through the column at a low
flow rate, which was followed by flushing of the column
with 400 ml of water, with a final backwash by upflow
with 600 ml of water.

Example 4

Regeneration of bioreactor

The packed column reactor employed in Example 1
was regenerated by passage of a hot (about 70°C) lye
solution (2% NaOH) through the column, until the regen-
erant outflow has a uniform light colour. The reactor
was rinsed with hot (about 70°C) distilled water until
the effluent had a pH of about 10.8, and neutralized
with a sodium pyrosulfite solution (0.5%) to a pH of
about 4.2. The reactor was rinsed with distilled water,
thereafter 800 ml of an MRS broth containing lactic
acid bacteria at 40°C were passed through the bed at a
low flow rate, which was followed by passage of 500 ml
of distilled water. About $2.5 \times 10^9$ bacteria/g dry
carrier were bound to the carrier.

Example 5

Bioconversion of lactic acid with *Rhizopus* mould

The selection of the carrier, filling of the col-
umn and sterilization of the system were performed in
accordance with Example 1.

*Rhizopus oryzae* was cultured on Potato Dextrose
agar plates (Difco) until the mould growth had sporu-
lated (about 5 days). A dilute spore suspension was
collected from the plates with sterile saline solution.
500 ml of this suspension at 30°C were passed through

12

the resin bed at a low flow rate, whereafter 500 ml of distilled water were passed through the bed.

The temperature of the bioconversion was 30°C. A feed solution was passed through the bioreactor at a rate of 2 l/h (10 bed volumes/h). The feed solution had the following composition:

| | |
|---|---|
| glucose | 10 g/l |
| $MgSO_4$ | 0.05 g/l |
| $K_2HPO_4$ | 0.1 g/l |
| $(NH_4)_2SO_4$ | 0.1 g/l |
| pH | 6.5 |

The productivity of the bioreactor was 2 g lactic acid/l bed volume/h. The reactor was run continuously for six weeks.

Example 6

Bioconversion of gluconic acid with *Aspergillus* mould

The selection of the carrier, filling of the column and sterilization of the system were performed in accordance with Example 1.

*Aspergillus niger* was cultured on Potato Dextrose agar plates (Difco) until the mould growth had sporulated (about 6 days). A dilute spore suspension was collected from the plates with sterile saline solution. 500 ml of this suspension at 30°C were passed through the resin bed at a low flow rate, whereafter 500 ml of distilled water were passed through the bed.

The temperature of the bioconversion was 30°C. A feed solution was passed through the bioreactor at a rate of 2 l/h (10 bed volumes/h). The feed solution was saturated with oxygen in an intermediate vessel prior to its passage into the bioreactor. The feed solution had the following composition:

| | |
|---|---|
| glucose | 10 g/l |
| corn steep liquor | 1.0 g/l |

13

| | |
|---|---|
| urea | 0.1 g/l |
| $MgSO_4$ | 1.0 g/l |
| $KH_2HPO_4$ | 0.1 g/l |
| $(NH_4)_2HPO_4$ | 0.1 g/l |
| pH | 6.5 |

The productivity of the bioreactor was 0.8 g gluconic acid/l bed volume/h. The reactor was run continuously for four weeks.

Example 7

Bioconversion of itaconic acid with *Aspergillus* mould

The selection of the carrier, filling of the column and sterilization of the system were performed in accordance with Example 1. Furthermore, the bioreactor was provided with a gas distribution ring below the carrier bed.

*Aspergillus terreus* was cultured on Czapek agar plates (Difco) until the mould growth had sporulated (about 5 days). A dilute spore suspension was collected from the plates with sterile saline solution. 500 ml of this suspension at 35°C were passed through the resin bed at a low flow rate, whereafter 500 ml of distilled water were passed through the bed.

The temperature of the bioconversion was 35°C. A feed solution was passed through the bioreactor at a rate of 2 l/h (10 bed volumes/h). The feed solution was saturated with oxygen in an intermediate vessel prior to its passage into the bioreactor. Moreover, oxygen was passed through the bed via a gas distribution ring below the bed at a rate of 200 ml/h (1 bed volume/h). The feed solution had the following composition:

| | |
|---|---|
| sucrose | 10 g/l |
| $ZnSO_4$ | 1.0 g/l |
| $MgSO_4$ | 3.0 g/l |

14

| CuSO$_4$ | 0.01 g/l |
| pH | 4.0 |

The productivity of the bioreactor was 0.6 g ita-
conic acid/l bed volume/h. The reactor was run continu-
ously for three weeks.

Example 8

Bioconversion of gluconic acid with *Aspergillus*
mould

The bioreactor employed was a 1.5 litre airlift
reactor (Braun Melsungen AG) equipped with a central
draft tube. The carrier in the reactor was maintained
in fluidized state by passing sterile air beneath the
draft tube. The feed solution was introduced to the
bottom part of the reactor, and the outlet was taken
from the clear top part of the reactor. The carrier em-
ployed was a granular DEAE cellulose in accordance with
Example 1. The reactor was filled, the system steril-
ized and the mould immobilized in accordance with the
procedure set out below.

80 g (200 ml) of granular DEAE cellulose were
suspended in distilled water, and the suspension was
mixed periodically for 5 hours. The hydrated carrier
was transferred to a sterilized airlift reactor con-
taining 1 l of 70% ethanol. The carrier was sterilized
by stirring for 1 h, whereafter the carrier was flushed
by displacing the ethanol with distilled water. There-
after the reactor was filled with Potato Dextrose broth
(Difco).

*Aspergillus niger* was cultured on Potato Dextrose
agar plates (Difco) until the mould growth had sporu-
lated (about 6 days). A spore suspension was collected
from the plates with sterile saline solution. The reac-
tor was aseptically inoculated with 50 ml of this sus-
pension and incubated at 30°C with 2.0 l/min aeration
for 72 hours.

15

The temperature of the bioconversion was 30°C. A feed solution was passed through the bioreactor at a rate of 2 l/h, and the reactor was aerated at a rate of 2 l/min. The feed solution had the following composition:

| | |
|---|---|
| glucose | 10 g/l |
| corn steep liquor | 1.0 g/l |
| urea | 0.1 g/l |
| $MgSO_4$ | 1.0 g/l |
| $KH_2PO_4$ | 0.1 g/l |
| $(NH_4)_2HPO_4$ | 0.1 g/l |
| pH | 6.5 |

The productivity of the bioreactor was 0.5 g gluconic acid/l reactor volume/h. The reactor was run continuously for six weeks.

16

Claims:

1. A method for preparing an organic acid or its
salt by a continuous process, c h a r a c t e r -
i z e d  in that
    a feed solution is continuously passed into a
bioreactor containing microorganisms bound to a solid
carrier, the said carrier being substantially non-
compressible and comprising a continuous matrix or
grains having a large surface area, the said matrix or
grains comprising loosely bound microparticles or
microfibres that are chemically, adhesively or
mechanically bound to one another at at least some
contact points of the discrete microparticles or
microfibres,
    the acidic solution withdrawn from the bioreactor
is passed through a column of an anion exchanger regen-
erated with alkali metal hydroxide, to which column the
acid is bound,
    the feed solution withdrawn from the anion ex-
change column is recycled to the bioreactor, and
    at suitable intervals, the feed solution is dis-
placed by water and the anion exchange resin is regen-
erated with alkali metal hydroxide to recover the acid
as an alkali salt,
    whereafter, if acid is the desired end product,
the alkali metal salt solution is passed through a col-
umn of a cation exchanger in hydrogen ion form to yield
an organic acid.
    2. A method as claimed in claim 1, c h a r a c -
t e r i z e d  in that the microparticles or micro-
fibres comprise anion exchange resin.
    3. A method as claimed in claim 1, c h a r a c -
t e r i z e d  in that the microparticles or micro-

17

fibres are of cellulose or rayon or a derivative thereof.

4. A method as claimed in claim 3, c h a r - a c t e r i z e d in that the carrier is of diethyl-aminoethylene-modified cellulose (DEAE cellulose) in which the microparticles or microfibres are agglomerated with polystyrene.

5. A method as claimed in any one of the preceding claims, c h a r a c t e r i z e d in that the binding capacity of the carrier (cells/g of dry carrier) is $10^8$-$10^{12}$.

6. A method as claimed in any one of the preceding claims, c h a r a c t e r i z e d in that the bioreactor is a mixed tank, basket, fluidized bed, packed bed, or filter reactor.

7. A method as claimed in any one of the preceding claims, c h a r a c t e r i z e d in that the anion exchanger comprises at least two columns.

8. A method as claimed in any one of the preceding claims, c h a r a c t e r i z e d in that the cation exchanger comprises at least two columns.

9. A method for preparing an aqueous solution of lactate or lactic acid by a continuous process, c h a r a c t e r i z e d in that

a raw material-containing solution is continuously passed into a bioreactor containing lactic acid-producing microorganisms bound to a solid carrier, the said carrier being substantially non-compressible and comprising a continuous matrix or grains having a large surface area, the said matrix or grains comprising loosely bound microparticles or microfibres that are chemically, adhesively or mechanically bound to one another at at least some contact points of the discrete microparticles or microfibres,

18

the lactic acid-containing solution withdrawn
from the bioreactor is passed through a column of an
anion exchanger regenerated with alkali metal
hydroxide, to which column the acid is bound,

5          the sugar-containing solution withdrawn from the
anion exchange column is recycled to the bioreactor,
and

at suitable intervals, the sugar solution is dis-
placed by water and the anion exchange resin is regen-

10       erated with alkali metal hydroxide to recover the acid
as alkali metal lactate,

whereafter, if lactic acid is the desired end
product, the alkali metal lactate solution is passed
through a column of a cation exchanger in hydrogen ion

15       form to yield lactic acid.

10. A method as claimed in claim 9, c h a r -
a c t e r i z e d in that the microparticles or micro-
fibres comprise anion exchange resin.

11. A method as claimed in claim 9, c h a r -

20       a c t e r i z e d in that the microparticles or micro-
fibres are of cellulose or rayon or a derivative
thereof.

12. A method as claimed in claim 11, c h a r -
a c t e r i z e d  in that the carrier is of diethyl-

25       aminoethylene-modified cellulose (DEAE cellulose) in
which the microparticles or microfibres are agglomer-
ated with polystyrene.

13. A method as claimed in any one of the pre-
ceding claims, c h a r a c t e r i z e d  in that the

30       binding capacity of the carrier (cells/g of dry car-
rier) is $10^8$-$10^{12}$.

14. A method as claimed in any one of the pre-
ceding claims, c h a r a c t e r i z e d  in that the
anion exchanger comprises at least two columns.

15. A method as claimed in any one of the preceding claims, c h a r a c t e r i z e d in that the cation exchanger comprises at least two columns.

5     16. A method as claimed in any one of the preceding claims, c h a r a c t e r i z e d in that sugar, e.g. glucose, lactose, sucrose or xylose, or a sugar-containing solution, such as molasses, is employed as the raw material.

# INTERNATIONAL SEARCH REPORT

| International application No. |
| --- |
| PCT/FI 95/00277 |

## A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12P 7/56, C12P 7/40

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

IFIPAT, WPIDS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| --- | --- | --- |
| X | DD 285792 A5 (MARTIN-LUTHER-UNIVERSITÄT HALLE), 3 January 1991 (03.01.91) <br> -- | 1-16 |
| X | US 4467034 A (HARTMUT VOELSKOW ET AL), 21 August 1984 (21.08.84), see example 5 <br> -- | 1-16 |
| X | WO 9306226 A1 (TSAO, GEORGE, T.), 1 April 1993 (01.04.93), page 9 - page 10 <br> -- <br> -------- | 1-16 |

☐ Further documents are listed in the continuation of Box C.   ☒ See patent family annex.

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